Chemical Analysis of Vitamin A and Analogs

Honors Research Thesis

Presented in Partial Fulfillment of the Requirements for graduation
"with Honors Research Distinction in Chemistry" in the undergraduate colleges of
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by
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ABSTRACT

Vitamin A plays an important role in growth, vision, epithelial differentiation, immune function, and reproduction. However, vitamin A metabolites like retinoic acid (RA) pose many toxic effects in the body. Certain retinoid drugs like N-(4-hydroxyphenyl)retinamide (4-HPR) have shown promise treating epithelial cancers. Further research into the nonhydrolyzable analog, 4-hydroxybenzylretinone (4-HBR), have determined that it is just as potent but without any of the residual toxicity associated with RA. A new synthetic method for this drug was created, using a *para*-methyl benzyl phenyl ether as protecting group for the terminal phenol. Synthetic efficiency was also increased by the development of a larger scale synthesis for the expensive starting retinoid, retinal. This new method can successfully synthesize 4-HBR at a lower cost with good yields. This is useful for future chemical and biological studies of the retinoid.

ACKNOWLEDGEMENTS

I would first like to thank my research advisor, Dr. Robert Curley, for giving me the opportunity to research in his lab for the past two years. His knowledge and experience have helped develop not only my laboratory technique, but also my critical thinking and intellect. My research experience has been fantastic, due in large part to Dr. Curley's patient and constant guidance.

I would also like to thank Dr. Terry Gustafson for introducing me to the world of chemical research, as well as introducing me to Dr. Curley. Dr. Gustafson's rigorous analytical chemistry course was the best possible preparation for the critical thinking required in an independent research experience.

I would finally like to thank my family. But for their constant love and support I would not have been able to succeed at The Ohio State University.

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I. INTRODUCTION

Vitamin A is an essential lipid-soluble component found in certain foods. In the human body, vitamin A plays an important role in growth, vision, epithelial differentiation, immune function, and reproduction[1]. The most common forms are retinol and retinyl esters. Retinol can be reversibly converted in the body to the vitamin A aldehyde form, retinal, the chemical basis of vision in animals. The aldehyde form can be further oxidized, irreversibly, to retinoic acid (**Figure 1**). Retinoic acid (RA) is important for cell growth and development, and is also toxic at high levels. Toxic effects include mucocutaneous irritation, night blindness, hyperlipidemia, headache, bone loss, and other developmental effects[2]. RA affects epithelial cells through nuclear receptor proteins, which resulted in the use of retinoids as drugs for epithelial cancers and dermatological diseases[2].

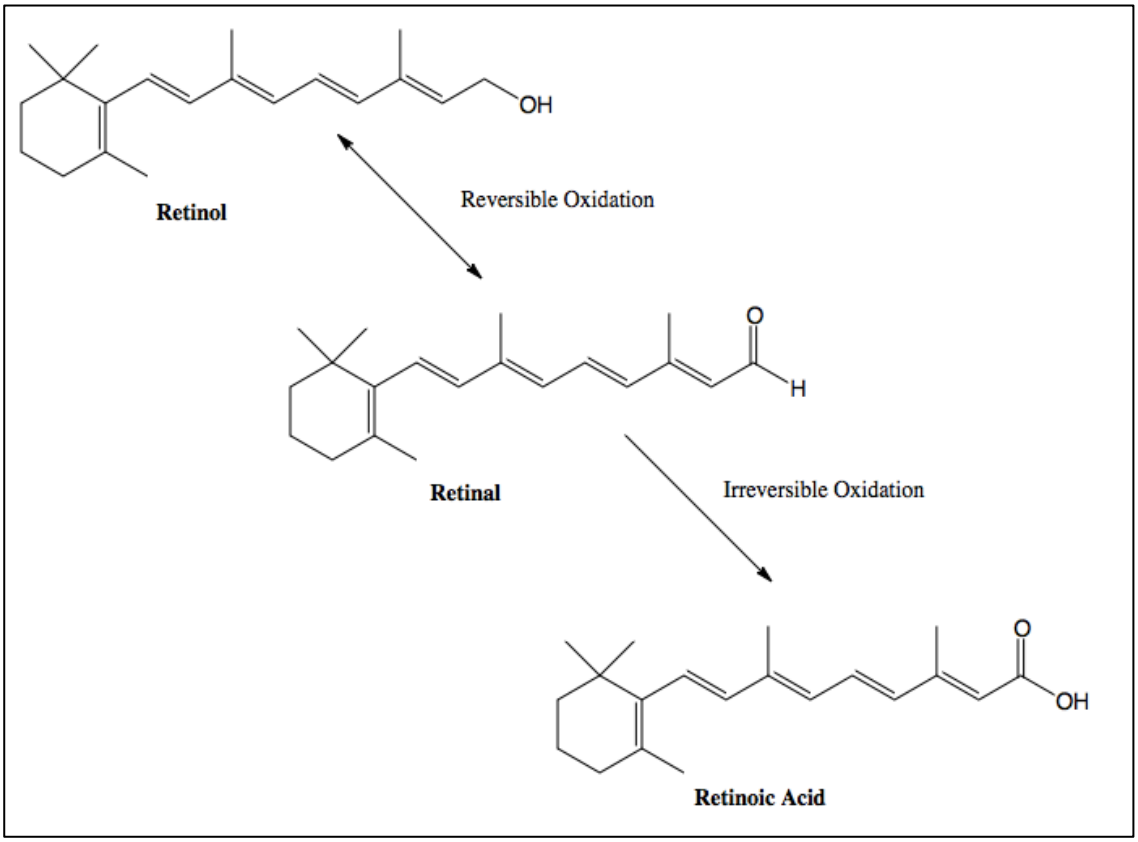


Figure 1: Vitamin A metabolism

Retinoids act, pharmacologically, to restore the regulation of differentiation and growth in malignant cells[3]. One drug thought to act in a RA-like manner is N-(4-hydroxyphenyl)retinamide (4-HPR; **1**). 4-HPR has been shown to induce apoptosis in a variety of cancer cell lines, including, breast, prostate, and leukemia[4]. Though similar to RA, 4-HPR is considerably less teratogenic, and consequently less toxic. However, 4-HPR has been shown to cause reversible ocular toxicity in human clinical trials. It has been determined that 4-HPR does not interact with the same nuclear receptor proteins as RA, and it is suggested that the toxic effects of 4-HPR may be a result of *in vivo* hydrolysis to RA[5]. This has led to interest in developing other synthetic retinoid analogs that address issues of toxicity.

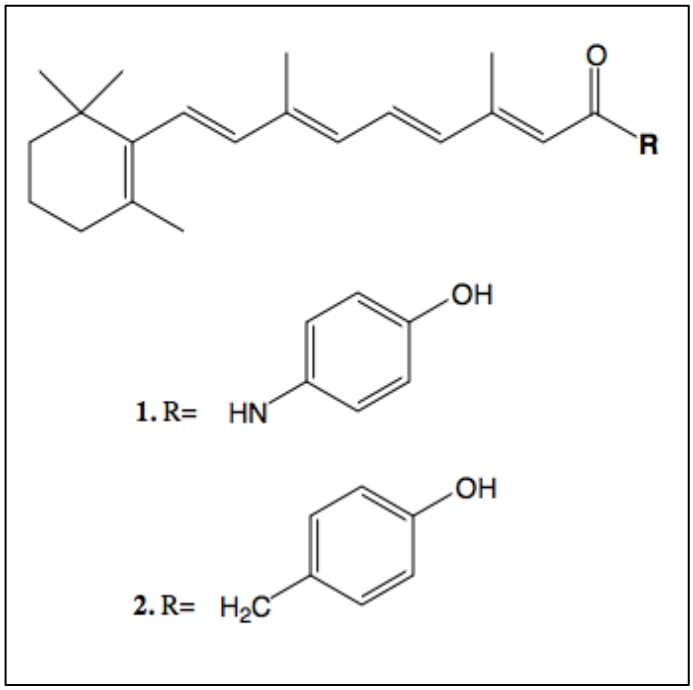


Figure 2: 4-HPR (**1**) and 4-HBR (**2**)

4-hydroxybenzylretinone (4-HBR; **2**) is a carbon-linked, nonhydrolyzable analogue of 4-HPR. The ketone, 4-HBR, like its amide analogue, has been shown to induce apoptosis and cell death in breast cancer cells[3]. 4-HPR and 4-HBR were compared in a study of mammary tumor growth in rats. The control animals had an average increase in tumor volume of 179% compared

with a decrease of 42% and 45% of rats treated with 4-HPR and 4-HBR, respectively[3]. An important difference between the two retinoids is, however, that 4-HBR is not hydrolyzed *in vivo* to liberate RA. This eliminates any residual RA-induced toxicity of 4-HBR, specifically the ocular toxicity associated with 4-HPR. 4-HBR therefore provides the ability to decrease tumor cell concentrations, while remaining less toxic at pharmacologically effective doses. In fact, researchers have determined that 4-HBR can be administered at doses nearly twice as high as 4-HPR without adverse teratogenic effects (unpublished results). It is now important to develop an efficient synthesis of 4-HBR for continued research related to its biochemical activity and effects.

II. DISCUSSION of Syntheses and Procedures

The necessity of 4-HBR for related chemical and biochemical studies has also led the desire for a more efficient synthetic route. The established procedure involves expensive starting materials and often produced 4-HBR in low yields. The *umpolung* synthesis began by activating retinal for reaction with trimethylsilyl cyanide, and then alkylating a suitable benzyl halide[5]. Silyl protecting groups were used both for the ketone and phenol during the reaction[6]. These protecting groups were removed with *tetra-n*-butylammonium fluoride (TBAF) with 65% yield. The original *umpolung* 4-HBR synthesis scheme is depicted in **Figure 3**.

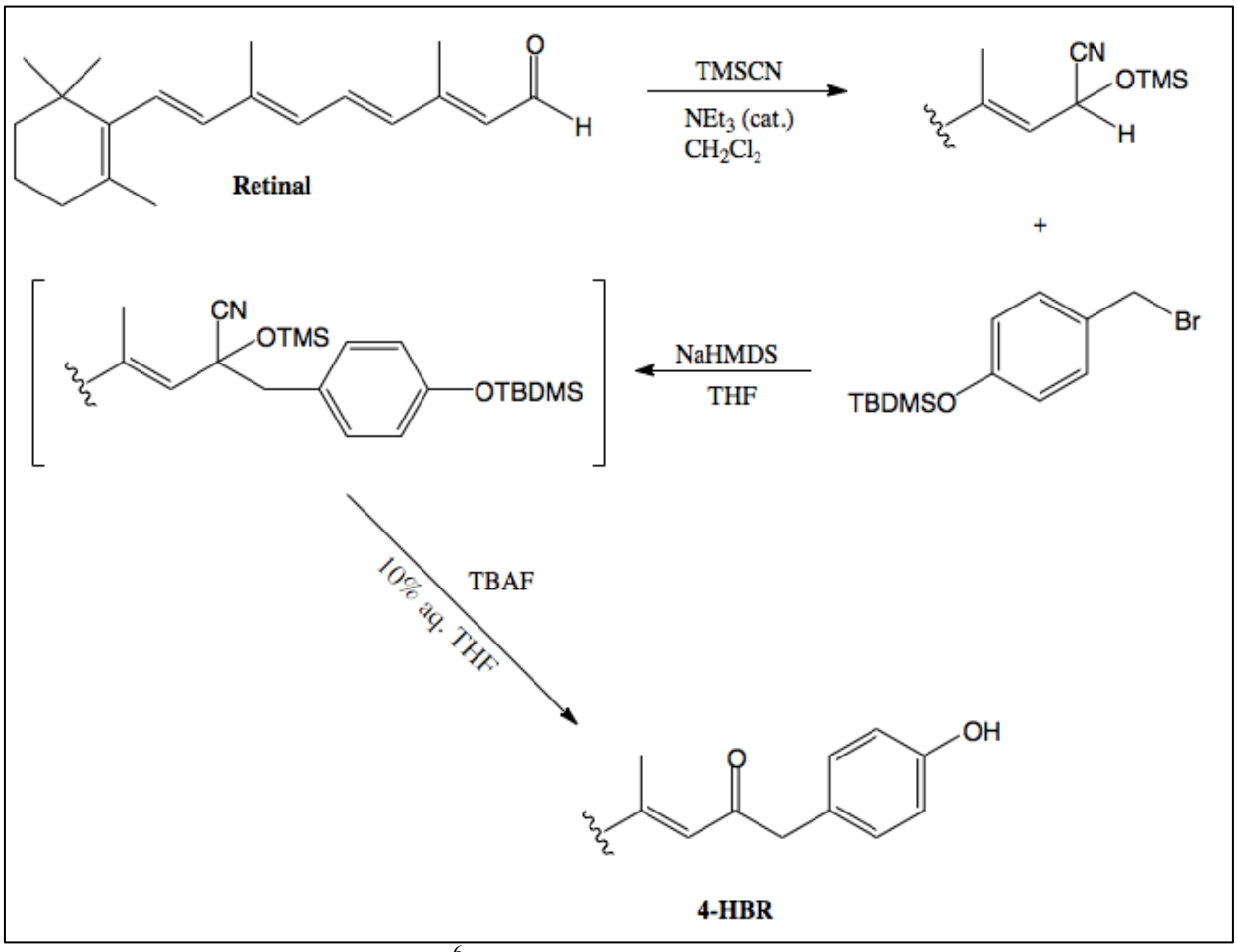


Figure 3: Original 4-HBR synthesis[6]

The focus of this work has been to increase synthetic efficiency, both by reducing cost and increasing reaction yields.

2.1 Retinal Synthesis Improvements and Applications

Vitamin A aldehyde is the retinoid used as the starting molecule for the 4-HBR synthesis. It is available commercially, however it is very expensive (~$200/g)[7]. It was therefore important to develop an efficient synthesis of this molecule on a reasonbly large scale (**Figure 4**). A retinal synthesis[7] was recently reported that starts from retinyl acetate, which is available at low cost (<$5/g) because of its production as a vitamin supplement. This synthesis proceeded with

transesterification of the acetate ester with methanol followed by oxidation of the obtained retinol upon passage through a column of MnO_2/diatomaceous earth. Although the yields were good, about 8% of the retinal obtained was the 13-*cis* isomer, which required careful chromatographic removal to give a solid product. It has been determined that the scaling of this oxidation column to handle greater than about 1 g of retinyl acetate results in a column of unwieldy proportions and slows elution of retinal sufficiently to result in a significant increase in the formation of side-products and in a reduction in yields.

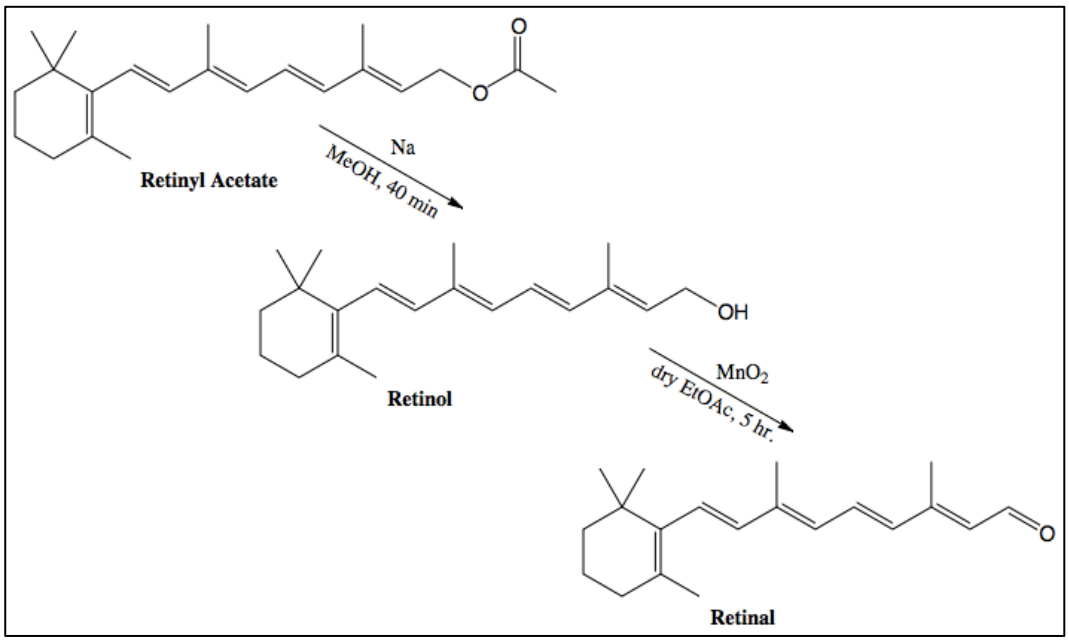


Figure 4: Retinal synthesis scheme

As previously reported, stirring retinyl acetate in a dilute anhydrous methanolic solution with one equivalent of sodium metal completes transesterification to retinol in forty minutes. We now recommend no volume reduction of the resulting solution before passage through an ion-exchange resin (Amberlite IRA-400, chloride form) to give a quantitative yield of the base labile retinol, which is used as obtained. The oxidation of retinol can be completed much more quickly (4.5-5.0 hours compared to 12-24 hours) when stirred in dry ethyl acetate with MnO_2 (25

eq). This synthetic route has been scaled up to 5 g of starting material with yields as good as 85% and also produced less 13-*cis* isomerization (only 2%, **Figure 5**). If desired, further purification can now be conducted by flash column chromatography on silica gel (5% ethyl acetate/hexane then 10% ethyl acetate/hexane).

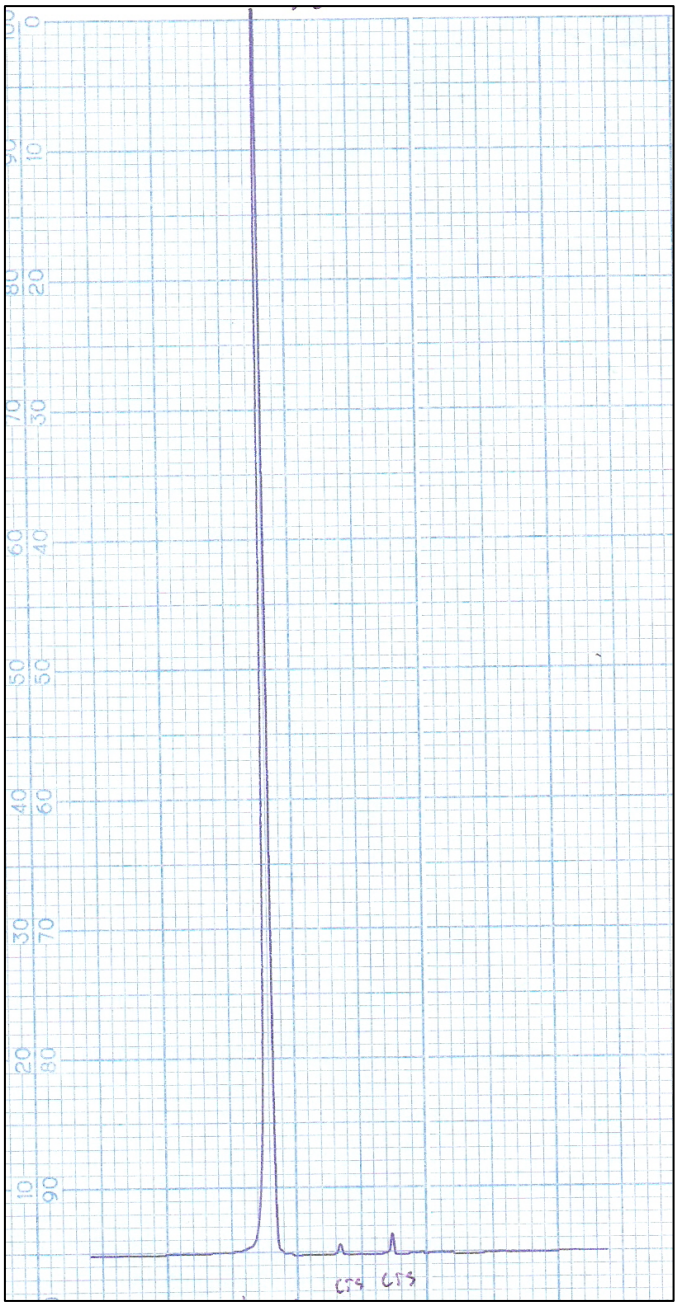


Figure 5: HPLC trace of retinal showing the major all-*trans* product and the minor *cis* isomers

At 40 times lower cost than of commercial retinal, this synthesis[8] can quickly produce larger quantities of the vitamin A aldehyde to be used in other synthetic reactions or for biological applications.

2.2 Benzyl Ether Protecting Group: Synthesis and Performance

Previous 4-HBR syntheses have relied on silyl ether protecting groups for both the aldehyde and the phenol group. Reactions yields were low, and often were associated with multiple side products. One significant side product formed during the benzyl alkylation and deprotection steps was the synthesis of a terminal benzyl phenyl ether (BPE;

Figure 6). The side product appeared to be due to *O*-alkylation of 4-HBR by residual benzylic bromide[6]. Investigation into alternate protecting groups suggested that a benzyl group could be used to protect the terminal phenol. A reaction scheme that targeted the molecule that was once a significant side product was thought to increase yields, specifically at the alkylation and deprotection steps of the synthesis.

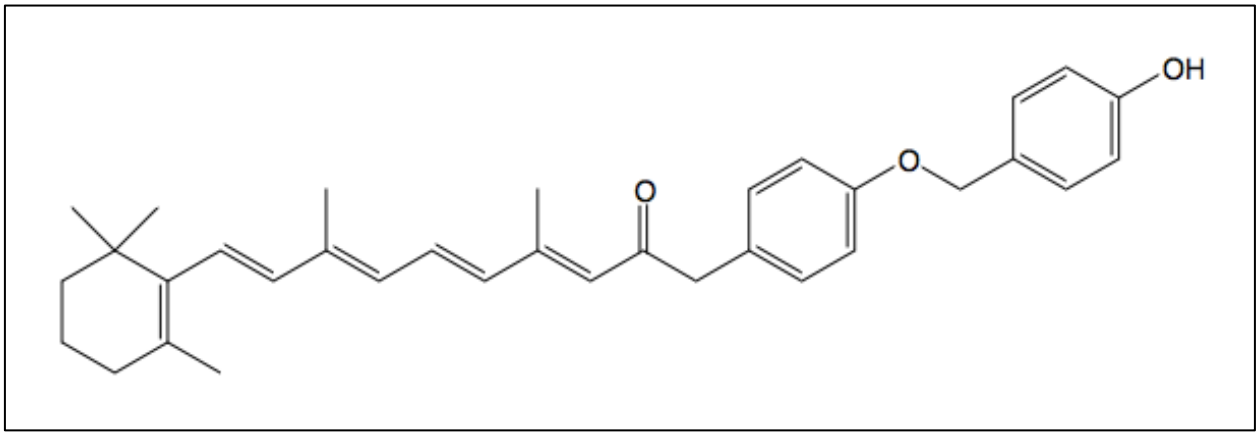


Figure 6: 4-HBR side product from alkylation and deprotection steps.

Trimethylsilyl iodide (TMSI) can be used to cleave ethers to their corresponding alcohols, however prolonged exposure to the reagent can lead to the formation of iodide products[11]. It was therefore important to determine the best benzyl protecting group in terms of rate of cleavage. There has not been substantial research on how substituted benzyl ethers affect cleavage rates. Two substituted BPEs were synthesized, along with the commercially available unsubstituted BPE, to determine the effect of electron donating and withdrawing groups on subsequent cleavage reactions. The decision was made to use weak electron withdrawing and weak electron donating groups because of the concern that TMSI may react more favorably with a stronger side chain (either donating or withdrawing), which would reduce cleavage yields. An un-substituted ether was used as a control molecule, and a *para*-methyl and a *para*-chloro group as weak electron donating and withdrawing groups, respectively (**Figure 7**).

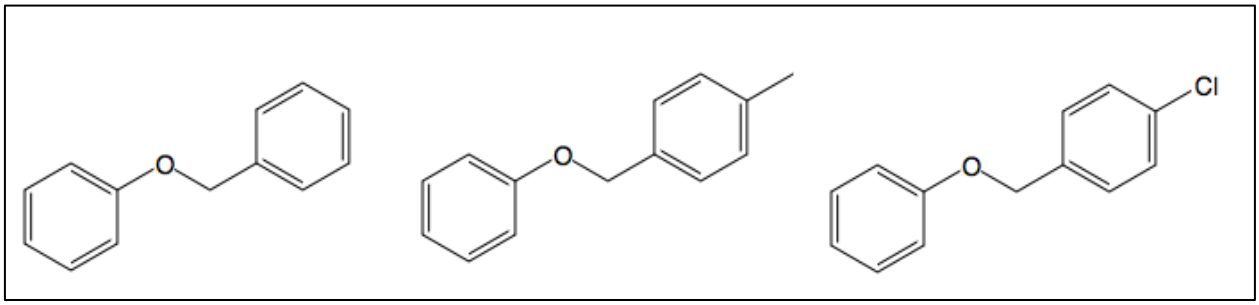


Figure 7: From left to right: BPE, *para*-methyl BPE, and *para*-chloro BPE.

The synthesized benzyl phenyl ethers were cleaved with TMSI and monitored for phenol production with an analytical high performance liquid chromatography (HPLC) method. A concern over whether the TMSI byproducts (I_2 and/or HI) would react with the polyene chain of the target retinoid (**2**) led to the inclusion of cyclohexene (10 eq) in the cleavage reaction scheme. Cyclohexene was also included in the comparative BPE cleavage reactions. Cyclohexene was used as a "dummy" electron rich double bond to intercept traces of HI or iodine in the reaction mixture, with the hope of reducing any acid-catalyzed or iodine-induced isomerization of the retinoid product. Reaction samples were taken at 10, 30, and 100 minutes, quenched with methanol and monitored at the maximum absorbance wavelength for phenol (270 nm)[10]. It was suspected that an electron-donating group would lead to greater reactivity and ether cleavage. This was the case, as the *para*-methyl BPE cleaved significantly faster than the other BPEs. The results for cleavage of the three benzyl phenyl ethers are shown in **Figure 8**.

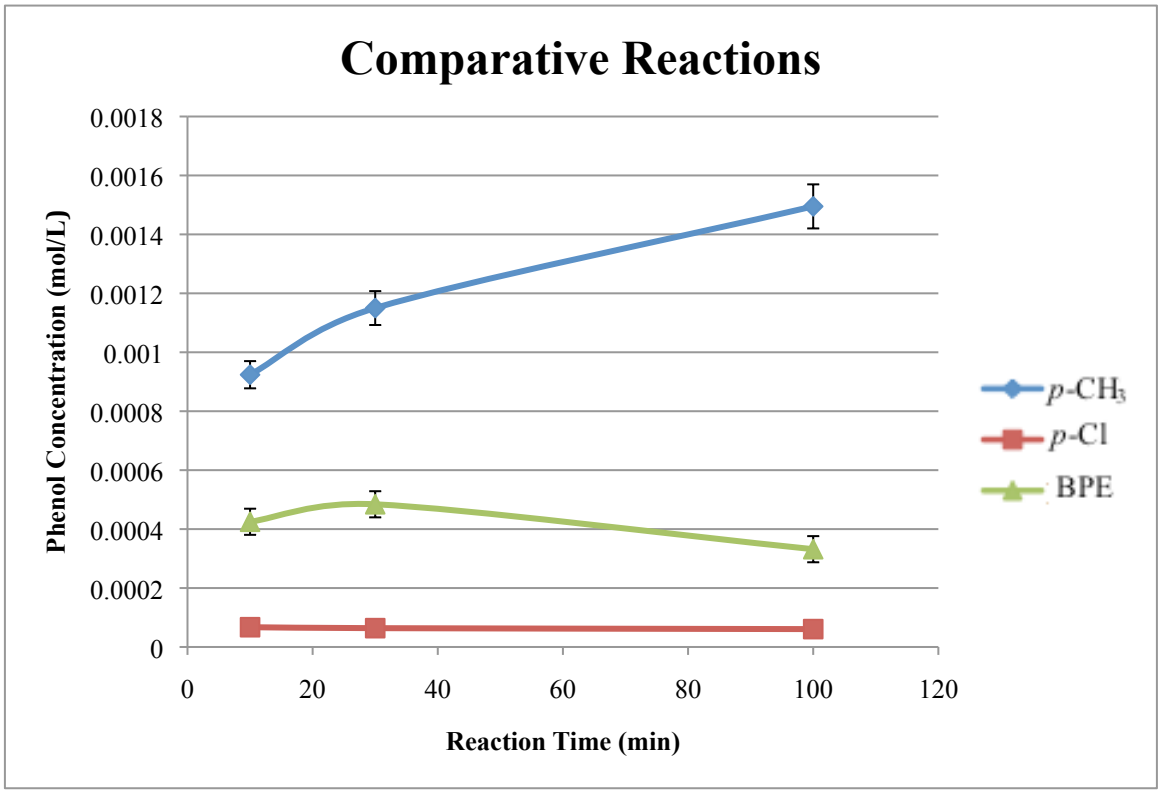


Figure 8: Phenol formation from cleavage of substituted BPEs

The much faster cleavage rate of the *para*-methyl BPE made it a logical choice for a benzyl protecting group because it would expose the product to TMSI for less time. The *para*-methyl BPE would become the group that would be connected via an alkylation reaction to the activated form of retinal.

2.3 *Umpolung* Synthesis of Protected 4-HBR

The synthesis of 4-HBR is based on alkylating a protected phenol group by the terminal end of retinal. The retinal must first be made more reactive; stirring with *tert*-butyldimethylsilyl cyanide protects both the carbonyl oxygen and attaches a cyano group to the carbonyl carbon. This dipole reversal results in an acidic hydrogen on the terminal end (**Figure 9**).

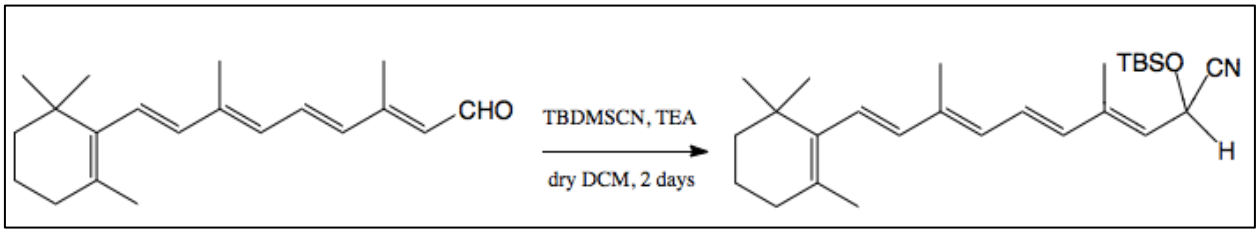


Figure 9: Retinal activation; carbonyl protection and dipole reversal.

The acidic hydrogen is removed with lithium hexamethyldisilazane (similar to **Figure 3**), and the protected phenol group (as a benzyl bromide; **Figure 10**) can be added. The bromide form of *para*-methyl BPE is synthesized from an aldehyde[9] via an alcohol intermediate[13] (**Figure 10**).

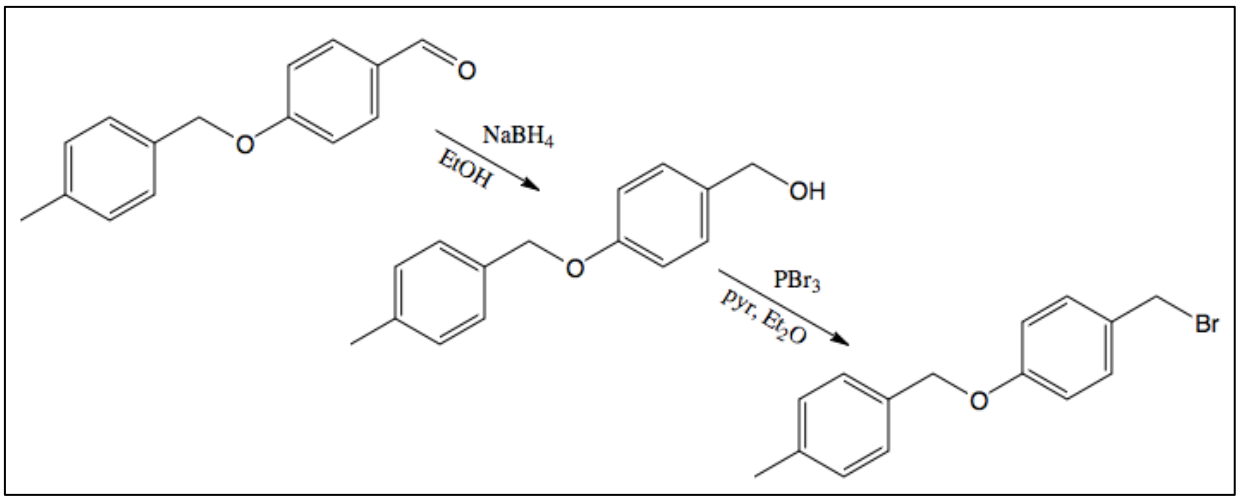


Figure 10: BPE protecting group reaction scheme

A portion of pure 4-HBR was used to attach the *para*-methyl BPE for comparison. The product structure (**Figure 11**) was confirmed by NMR and was used as a reference for the deprotection products.

2.4 Deprotection

The deprotection of 4-HBR involved two steps: removing the *tert*-butyldimethylsilyl (TBS) and cyano groups and removing the benzyl group. A portion of protected 4-HBR was

examined by first removing the TBS group. After one day stirring with *tetra-n*-butylammonium fluoride (TBAF), the product was successfully converted to the ketone form of protected 4-HBR (**Figure 11**). This targeted product is very similar to the prominent side product in the original reaction scheme (**Figure 6**). The product was then subjected to TMSI in an attempt to cleave the benzyl group.

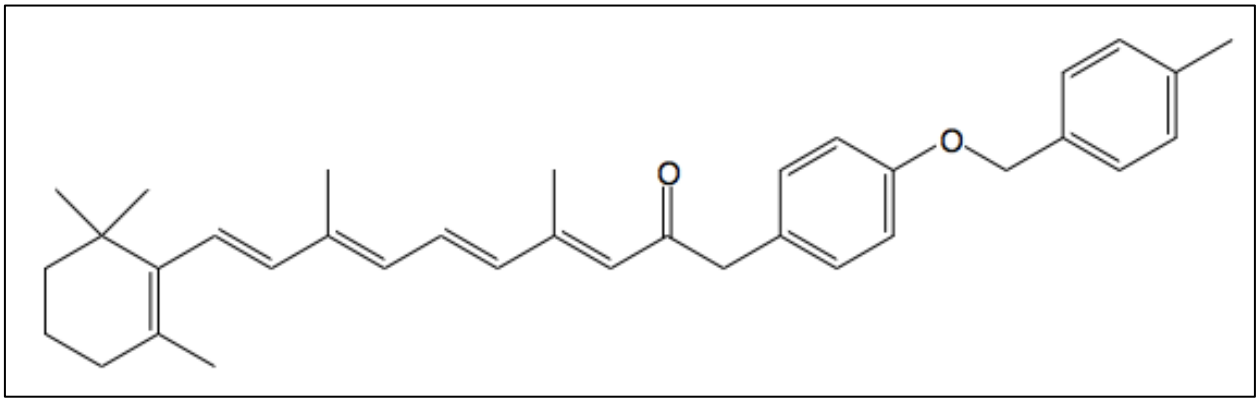


Figure 11: Benzyl protected, post-TBAF 4-HBR ketone.

As adapted from Kadota *et al.*, TMSI/Et_3N was first used in attempt to cleave the benzyl protecting group to the phenyl alcohol. Extended reaction times were required because of the chemical environment of the retinoid, and minimal reaction progress was noted. The product was then treated in a variety of chemical scenarios. The reactions were first run without cyclohexene, to address the possibility that this was slowing cleavage rates, at room temperature. Reaction samples were quenched and assayed in a similar method to the BPE cleavage comparisons (quenched with MeOH at 30, 60, and 90 minutes). The HPLC (85% methanol/water at 350 nm) was compared to that of pure 4-HBR. The HPLC results confirmed product synthesis, starting as early as the 30-minute mark (**Figure 12**). This suggests that TMSI can in fact cleave the protected retinoid to yield 4-HBR.

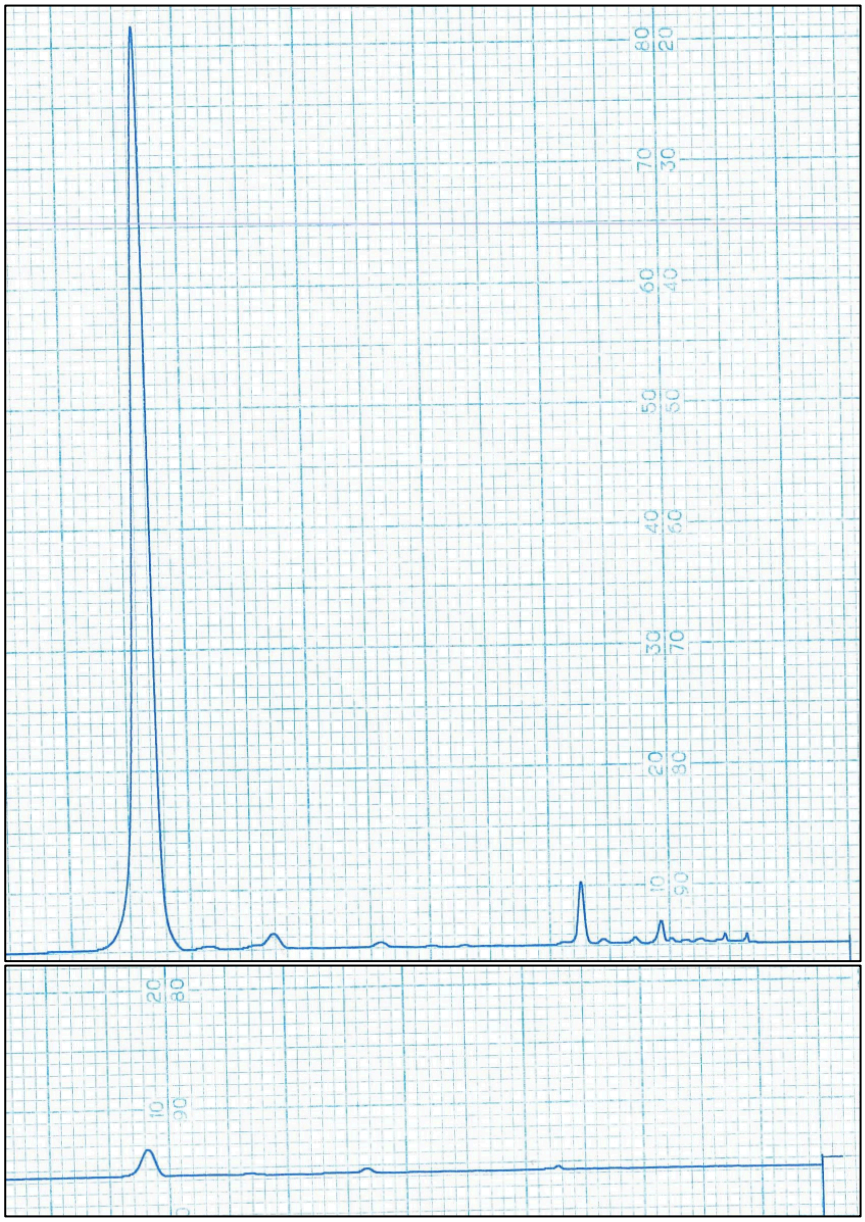


Figure 12: HPLC trace comparing pure 4-HBR (top) to the 30-minute TMSI cleavage product. 4-HBR retention time at 22.8 min.

2.5 4-HBR Synthesis Improvements

This project has successfully synthesized or isolated the following products: retinal, BPE, 4-methyl BPE, 4-chloro BPE, 4-(4-methylbenzyoxy)benzaldehyde, 4-(4-methylbenzyoxy)benzyl alcohol, 4-(4-methylbenzyoxy)benzyl bromide, activated retinal, protected 4-HBR, protected 4-HBR ketone. All products were used in the development of a new synthetic scheme for 4-HBR.

The overall cost of synthesis was greatly reduced by the retinal improvements. As the base retionoid for the 4-HBR synthesis, retinal is the most essential starting material. The synthetic developments to retinal have not only decreased its cost 40-fold, but also increased efficiency (85% yields) and product purity (98% all-*trans*).

Yields in the 4-HBR reaction scheme have been comparable to the previous synthesis[6]. However, the new synthesis addresses the most significant side product formed. Fine-tuning of the deprotection steps can make this step far more efficient than the established synthesis. Furthermore, the products synthesized in the new method have shown good molecular and isomer purity.

2.6 Future Directions

Continued investigation of potential phenol protecting groups could help futher improve the 4-HBR synthesis. Kadota *et al.* suggests that methoxyphenyl methyl (MPM) ethers can be selectively cleaved with TMSI/Et_3N. Investigation into the applications of an MPM ether protecting group on 4-HBR could illuminate an alternate synthetic route. However, the initial concerns of this type of chemical environment must be addressed. Although the literature suggests selective cleavage of MPM ethers, the presence of a methoxy ether may require additional equivalents of TMSI. Any excess of this reactant may lead to isomerization of the product retinoid, either from HI or iodine.

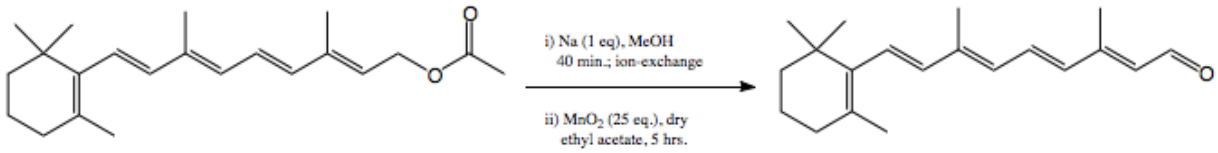
Additionally, there is not much known about the biological targets of 4-HBR. Studies have shown that 4-HBR binds poorly to the retinoic acid receptors (RAR) α, β, and γ [5]. The affinity for these receptors is similar to that of 4-HPR, however as discussed, 4-HBR cannot hydrolyze to liberate RA. A possible study could examine which cellular proteins interact with

4-HBR. A specially designed affinity gel containing 4-HPR/4-HBR could be used to identify binding proteins to the drug and provide important insights to its cellular metabolism. This may illuminate the chemotherapeutic mechanisms of these retinoid pharmaceuticals.

III. EXPERIMENTAL

All reagents were purchased as reagent grade from Sigma-Aldrich (Milwaukee, WI) and were used as obtained. Reactions were performed in oven-dried glassware under an argon atmosphere and gold fluorescent lights. Analytical TLC was performed on silica gel 60 F254 aluminum-backed plates from Merck (Darmstadt, Germany). Flash column chromatography was performed on silica gel 60 (230-400 mesh) from Merck. Analysis by HPLC was done on a Beckman Instruments unit (model 127 pump, model 166 detector) using 1 mL/min of MeOH-H_2O through a Polaris C18 column. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker Instruments DRX400 spectrometer (Billerica, MA) operating at 400 MHz for ^{1}H measurements. Electrospray mass spectra were measured on a Micromass QTOF mass spectrometer in the Ohio State University Campus Chemical Instrument Center.

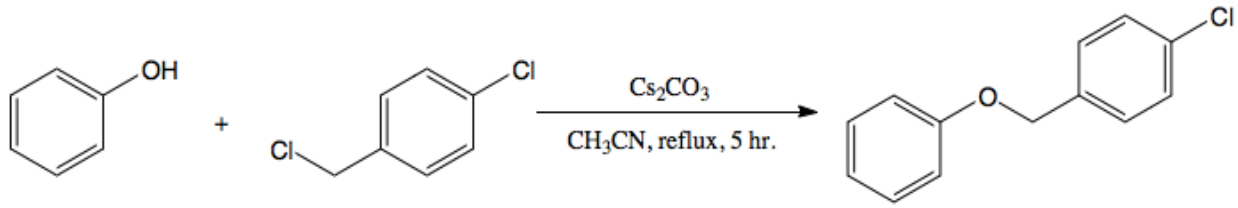
Retinal



To a stirred solution of retinyl acetate (5.10 g; 15.5 mmol) in 500 mL of anhydrous methanol was added sodium pieces (0.39 g; 1.1 eq.) and the mixture stirred for 40 minutes at which time TLC (20% ethyl acetate/hexane) indicated complete consumption of retinyl acetate. The solution was passed through Amberlite IRA-400 ion-exchange resin (6.5 g; chloride form) and the

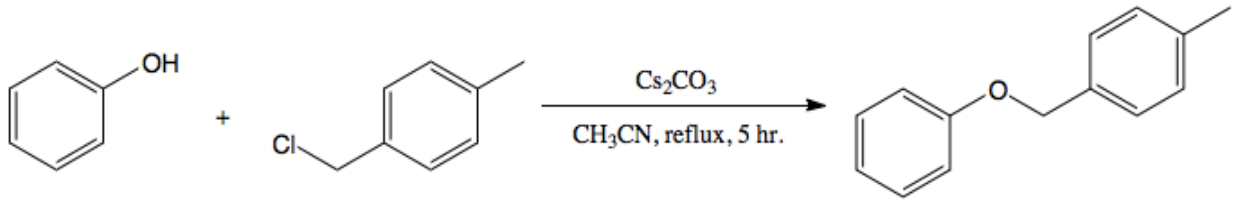
column rinsed with methanol. The eluent was concentrated under reduced pressure to a red oil which was dissolved in the minimum amount of dry dichloromethane (2-3 mL). After addition of activated MnO_2 (33 g; 24.4 eq.) and dry ethyl acetate (40 ml), the thick suspension was stirred for 5 hr. at which time TLC indicated complete oxidation to retinal. The product solution was filtered through a pad of diatomaceous earth with dichloromethane rinsing and the filtrate was evaporated to give solid yellow-orange retinal; (3.75 g; 85% yield): mp 57.5-60 °C; HPLC: t_R = 10.3 min (~96%). ^{1}H NMR ($CDCl_3$): δ 0.93 (s, 6H, CMe_2), 1.36 (m, 2H, CH_2), 1.51 (m, 2H, CH_2), 1.61 (s, 3H, CH_3), 1.90 (m, 2H, CH_2), 1.91 (s, 3H, CH_3), 2.20 (s, 3H, CH_3), 5.83 (d, 1H, *J* = 8 Hz, 14-CH), 6.03-6.25 (m, 4H, vinyls), 7.03 (dd, 1H, *J* = 11.6 and 14.9 Hz, 12-CH), 9.97 (d, 1H, *J* = 8 Hz, CHO). ^{13}C NMR ($CDCl_3$): δ 13.6, 13.7, 19.9, 22.4, 29.7, 33.8, 34.9, 40.3, 129.7, 130.19, 130.21, 130.96, 133.1, 135.3, 137.8, 138.3, 141.7, 155.1, 191.3. HRMS (ESI): *m/z* [M + Na]$^+$: Calcd for $C_{20}H_{28}O$ + Na: 307.2038. Found: 307.2040.

4-chloro benzyl phenyl ether



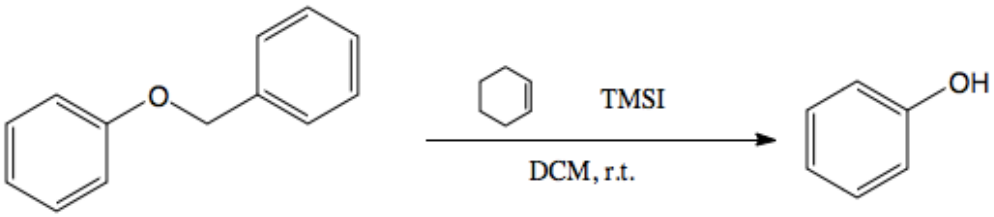
Phenol (0.1016 g, 1.08 mmol) and cesium carbonate (0.69 g, 2 eq.) were dissolved in 40 mL acetonitrile. *para*-Chlorobenzyl chloride (0.85 g, 5.3 eq.) was added and the mixture was stirred at reflux for 5 hr. Solvent was evaporated and organic product extracted in ether from water (3 x 50 mL). The product was washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure to yield 4-chloro benzyl phenyl ether as a white solid (19.5 mg, 8.25 %). ^{1}H NMR ($CDCl_3$): δ 5.02 (s, 2H, CH_2), 6.93-7.35 (m, 9H, Ar).

4-methyl benzyl phenyl ether



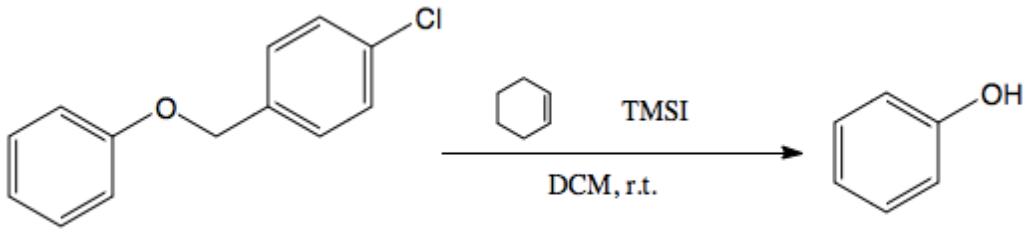
Phenol (0.1002 g, 1.06 mmol) and cesium carbonate (0.47 g, 1.5 eq.) were dissolved in 40 mL acetonitrile. 4-methylbenzyl chloride (0.70 mL, 0.75 g, 5.3 eq.) was added and the mixture was stirred at reflux for 5 hr. Solvent was evaporated and organic product extracted in ether from water (3 x 50 mL). The product was washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure to yield 4-methyl benzyl phenyl ether as a white solid (7.5 mg, 4.0 %). ^{1}H NMR ($CDCl_3$): δ 2.36 (s, 3H, CH_3), 5.02 (s, 2H, CH_2), 6.96-7.34 (m, 9H, Ar).

Benzyl phenyl ether Cleavage



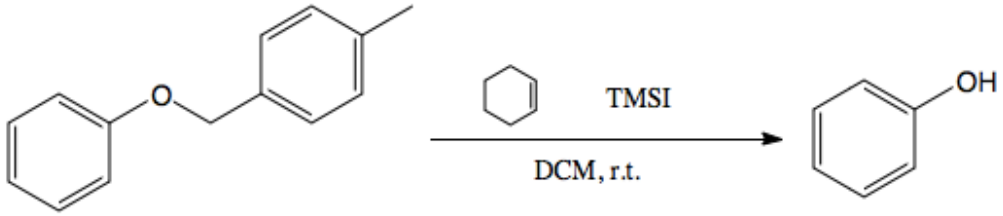
Benzyl phenyl ether (7.04 mg, 0.038 mmol) and cyclohexene (25 mg, 8 eq) were combined in dichloromethane (25 mL). Trimethylsilyl iodide (19.1 mg, 2.5 eq) was added and the reaction mixture was stirred at room temperature. At 10, 30, and 100 min., reaction progress was monitored by removing 2 mL of reaction mixture and quenching with MeOH (2 mL). The quenched product was analyzed on by HPLC (70% methanol/water) at 270 nm. The absorbances were compared to a standard phenol curve to determine product concentration.

4-chloro benzyl phenyl ether Cleavage



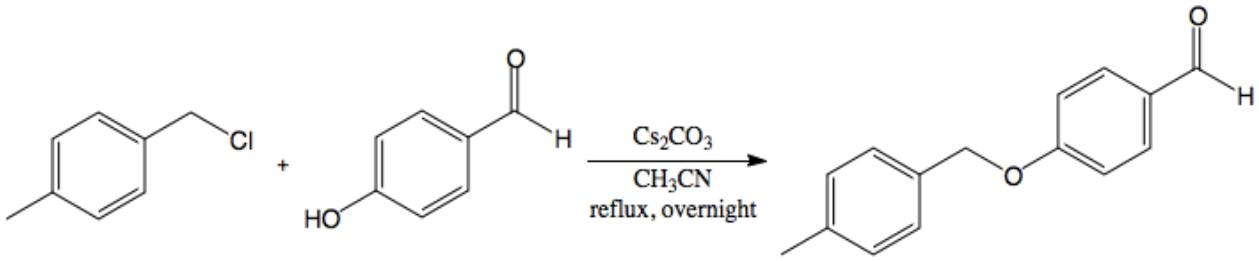
4-Chloro benzyl phenyl ether (8.3 mg, 0.038 mmol) and cyclohexene (25 mg, 8 eq) were combined in dichloromethane (25 mL). Trimethylsilyl iodide (19.1 mg, 2.5 eq) was added and the reaction mixture was stirred at room temperature. At 10, 30, and 100 min., reaction progress was monitored by removing 2 mL of reaction mixture and quenching with MeOH (2 mL). The quenched product was analyzed on by HPLC (70% methanol/water) at 270 nm. The absorbances were compared to a standard phenol curve to determine product concentration.

4-methyl benzyl phenyl ether Cleavage



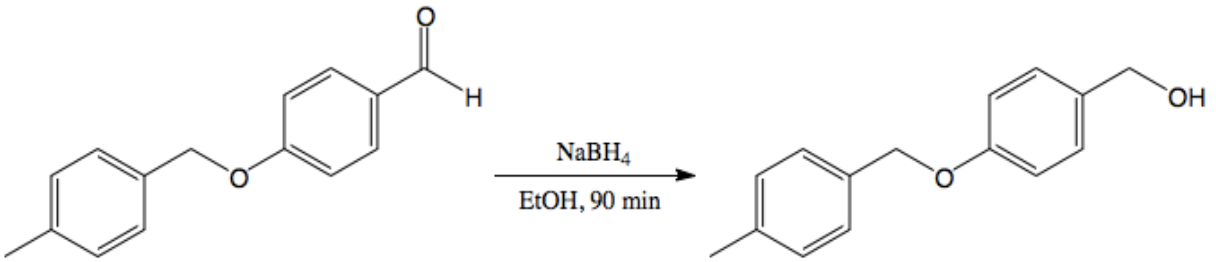
4-Methyl benzyl phenyl ether (7.5 mg, 0.038 mmol) and cyclohexene (25 mg, 8 eq) were combined in dichloromethane (25 mL). Trimethylsilyl iodide (19.1 mg, 2.5 eq) was added and the reaction mixture was stirred at room temperature. At 10, 30, and 100 min., reaction progress was monitored by removing 2 mL of reaction mixture and quenching with MeOH (2 mL). The quenched product was analyzed on by HPLC (70% methanol/water) at 270 nm. The absorbances were compared to a standard phenol curve to determine product concentration.

4-(4-Methylbenzyloxy)benzaldehyde



4-Hydroxybenzaldehyde (6.60 g, 54.0 mmol) and 4-methylbenzylchloride (5.0 g, 35.6 mmol) were added to a stirred solution of Cs_2CO_3 (23.14 g, 71.0 mmol) in 100 mL dry acetonitrile. The mixture was stirred at reflux for 48 hr. Solvent was evaporated under air overnight and the product was extracted into ether from 1 M NaOH and washed with brine. The organic layer was filtered, dried with $MgSO_4$, then evaporated under reduced pressure to yield the crude 4-(4-methylbenzyloxy)benzaldehyde as a white solid (7.8712 g, 97%). ^{1}H NMR ($CDCl_3$): δ 2.34 (s, 3H, CH_3), 5.07 (s, 2H, CH_2), 7.02-7.30 (m, 8H, Ar), 9.84 (s, 1H, CHO). HRMS (ESI): *m/z* [M + Na]$^+$: Calcd for $C_{15}H_{14}O_2$ + Na: 249.0891. Found: 249.0883.

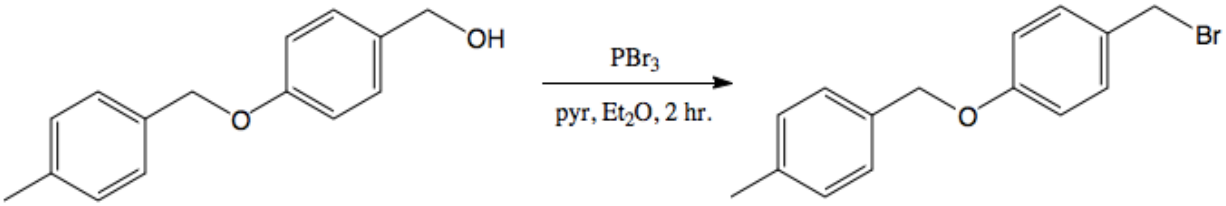
4-(4-Methylbenzyloxy)benzyl aclohol



4-(4-Methylbenzyloxy)benzaldehyde (3.87 g, 17.1 mmol) was dissolved in anhydrous ethanol containing sodium borohydride (1.31 g, 2 eq.). The reaction mixture was stirred at r.t. for 90 min. and then quenched with saturated ammonium chloride solution (15 mL). The organic product was then extracted into ethyl acetate from water (3 x 100 mL). The product was washed with brine and dried over $MgSO_4$, and solvent was evaporated under reduced pressure. Column

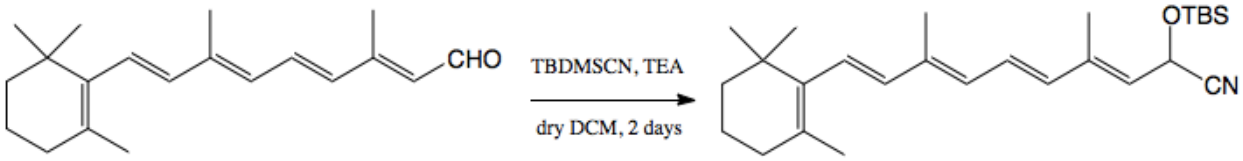
chromatographic purification (20% ethyl actetate/hexane then 40% ethyl acetate/hexane) yielded 4-(4-methylbenzyloxy)benzyl alcohol as a white solid (2.92 g, 74.7%). ^{1}H NMR ($CDCl_3$): δ 2.34 (s, 3H, CH_3), 4.595 (brd, 2H, C**H**$_2$OH, *J*= 2.03 Hz), 5.01 (s, 2H, CH_2), 6.93-7.31 (m, 8H, Ar). ^{13}C NMR ($CDCl_3$): δ 21.95, 65.86, 70.77, 115.74, 116.02, 127.63, 128.33, 129.34, 130.03, 134.67, 138.51. HRMS (ESI): *m/z* [M + Na]$^+$: Calcd for $C_{15}H_{16}O_2$ + Na: 251.1048. Found: 251.1051.

4-(4-Methylbenzyloxy)benzyl bromide



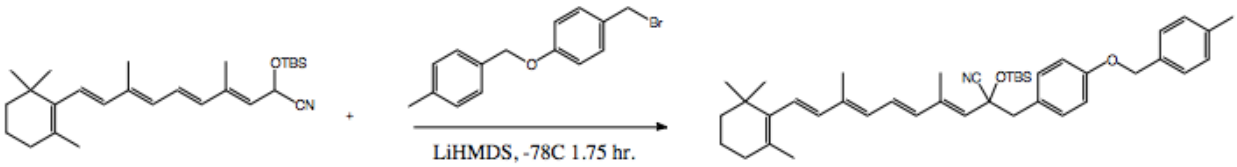
4-(4-Methylbenzyloxy)benzyl alcohol (0.99 g, 4.34 mmol) and pyridine (.08 mL) were dissolved in 15 mL of Et_2O and cooled to 0 °C. PBr_3 (1.29 g, 0.45 mL, 4.78 mmol) was added in 10 mL of Et_2O over 30 min. The reaction mixture was then stirred at r.t. for 90 min. The organic product was extracted into ether from ice/brine mixture. The organic layer was washed with saturated $NaHCO_3$, brine, and dried over $MgSO_4$. The solution was filtered and evaporated under reduced pressure to give 4-(4-methylbenzyloxy)benzyl bromide as a white solid (1.06 g, 83.8 %) which was used as obtained. ^{1}H NMR ($CDCl_3$): δ 2.34 (s, 3H, CH_3), 4.47 (s, 2H, CH_2), 4.99 (s, 2H, CH_2), 6.89-7.29 (m, 8H, Ar). ^{13}C NMR ($CDCl_3$): δ 21.95, 34.68, 70.82, 115.90, 116.12, 128.34, 130.11, 130.88, 131.20, 134.44, 138.63.

Retinal Activation



Retinal (1.0 g, 3.5 mmol) was combined with *tert*-butyldimethylsilyl cyanide (1.76 g, 12.7 mmol) and triethylamine (0.2 mL, 1.27 mmol) in dry dichloromethane. The reaction mixture was stirred at r.t. for 2 days. TLC (20% ethyl acetate/hexane) confirmed reaction completion, and the sample was purified via column chromatography (5% ethyl acetate/hexane). The solvent was evaporated under reduced pressure to give activated retinal as a red semisolid (1.41 g, 94%) which was used as soon as possible in the next reaction.

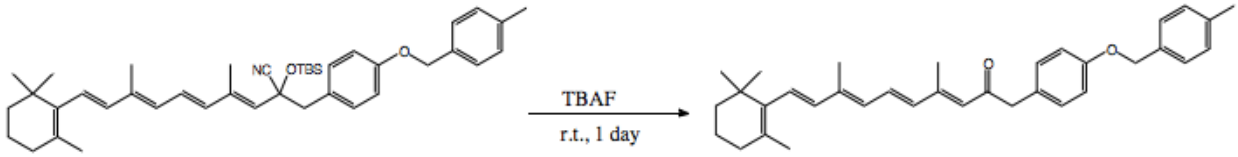
Alkylation



Activated retinal (1.41 g, 3.31 mmol) was dissolved in 20 mL dry THF and cooled to -78 °C. Lithium hexamethyldisilazane (1 M in THF, 5.0 mL) was added and the mixture was stirred for 30 min. 4-(4-Methylbenzyloxy)benzyl bromide (1.05 g, 1.1 eq) was added in dry THF (20 mL) and reaction was stirred at -78 °C for 1.75 hr. The reaction was warmed to r.t. as stirred for 15 min, and then was quenched with saturated NH_4Cl (10 mL) and H_2O (10 mL). Organic product was extracted into ethyl acetate from water (3 x 200 mL) and washed with brine. The organic layer was dried over Na_2SO_4, filtered and concentrated to give crude product as a red semisolid. Column chromatography (2.5% ethyl acetate/hexane) yielded pure product as a yellow-orange crystalline solid (650 mg, 31 %). ^{1}H NMR ($CDCl_3$): δ 0.08 (s, 3H, CH_3), 0.11 (s, 3H, CH_3), 0.85 (s, 9H, CH_3), 1.01 (s, 6H, CH_3), 1.44-1.46 (m, 2H, CH_2), 1.55 (s, 3H, CH_3), 1.58-1.66 (m, 2H,

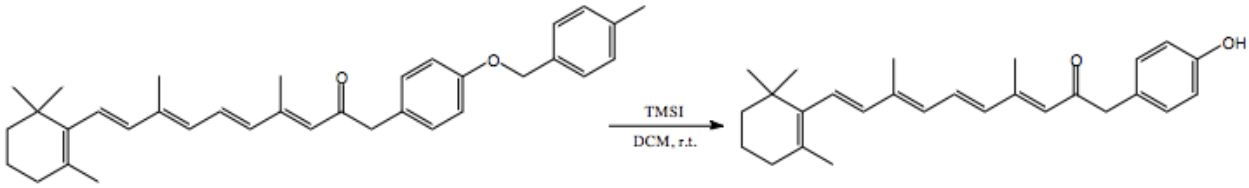
CH_2), 1.69 (s, 3H, CH_3), 1.95 (s, 3H, CH_3), 2.00 (m, 2H, CH_2), 2.34 (s, 3H, CH_3), 3.06 (d, 2H, CH_2) 4.99 (s, 2H, CH_2), 5.38 (s, 1H, 14-CH), 6.06-6.17 (m, 4H, vinyls), 6.65 (dd, 1H, *J* = 11.4 and 11.3 Hz, 11-CH), 6.88 (d, 2H, *J* = 8.5 Hz, Ar), 7.15-7.18 (m, 4H, Ar), 7.29 (d, 2H, *J* = 7.9 Hz, Ar). HRMS (ESI): *m/z* $[M + Na]^+$: Calcd for $C_{42}H_{57}O_2SiN$ + Na: 658.4056. Found: 658.4033.

Silyl Deprotection



Protected 4-HBR (50 mg, 0.079 mmol) was stirred with *tetra-n*-butylammonium fluoride (22.6 mg, 1.0 eq) in 90% THF/water overnight. TLC (20% ethyl acetate/hexanes) confirmed reaction completion and after volume reduction the product was extracted into ethyl acetate from water. The organic layer was washed with brine and dried over $MgSO_4$. Preparative TLC (20% ethyl acetate/hexanes) was used to provide pure 4-methylbenzyl protected 4-HBR as a yellow crystalline solid (12 mg, 31%). ^{1}H NMR ($CDCl_3$): δ 1.02 (s, 6H, CMe_2), 1.45-1.48 (m, 2H, CH_2), 1.60-1.63 (m, 2H, CH_2), 1.71 (s, 3H, CH_3), 1.98 (s, 3H, CH_3), 2.02 (m, 2H, CH_2), 2.32 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 3.66 (s, 2H, CH_2), 4.99 (s, 2H, CH_2), 6.11-6.31 (m, 5H, vinyls), 7.03 (dd, 1H, *J* = 11.4 and 11.5 Hz, 11-CH), 6.92 (d, 2H, *J* = 8.4 Hz, Ar), 7.12 (d, 2H, *J* = 8.4 Hz, Ar), 7.17 (d, 2H, *J* = 7.8 Hz, Ar), 7.30 (d, 2H, *J* = 7.8 Hz, Ar). ^{13}C NMR ($CDCl_3$): δ 13.71, 15.04, 20.00, 21.96, 22.52, 29.74, 33.91, 35.05, 40.40, 51.70, 70.75, 115.85, 125.77, 128.18, 128.38, 129.76, 130.02, 130.39, 130.90, 131.23, 133.16, 134.81, 136.40, 138.02, 138.46, 138.49, 140.76, 153.08, 158.62, 199.42. HRMS (ESI): *m/z* $[M + Na]^+$: Calcd for $C_{35}H_{42}O_2$ + Na: 517.3083. Found: 517.3077.

Benzyl Deprotection



4-Methylbenzyl protected 4-HBR (13 mg, 0.026 mmol) was stirred with trimethylsilyl iodide (10 μL, 2.7 eq) in dichloromethane at r.t. At 30, 60, and 90 min., reaction progress was monitored by removing 10 μL of reaction mixture and quenching with MeOH (50 μL)). The quenched product was analyzed by HPLC (85% methanol/water) at 350 nm. The product retention time was compared to that of pure 4-hydroxybenzylretinone to confirm the formation of the product retinoid. HPLC: t_R = 22.8 min.

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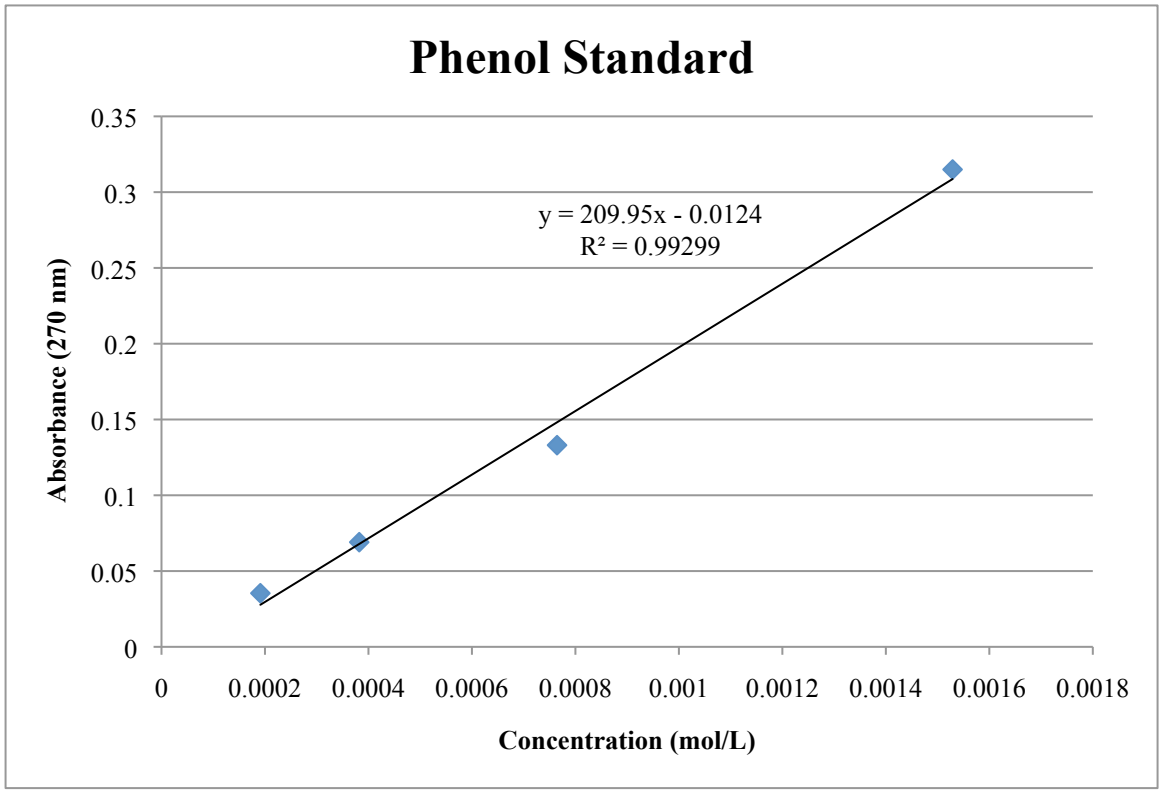
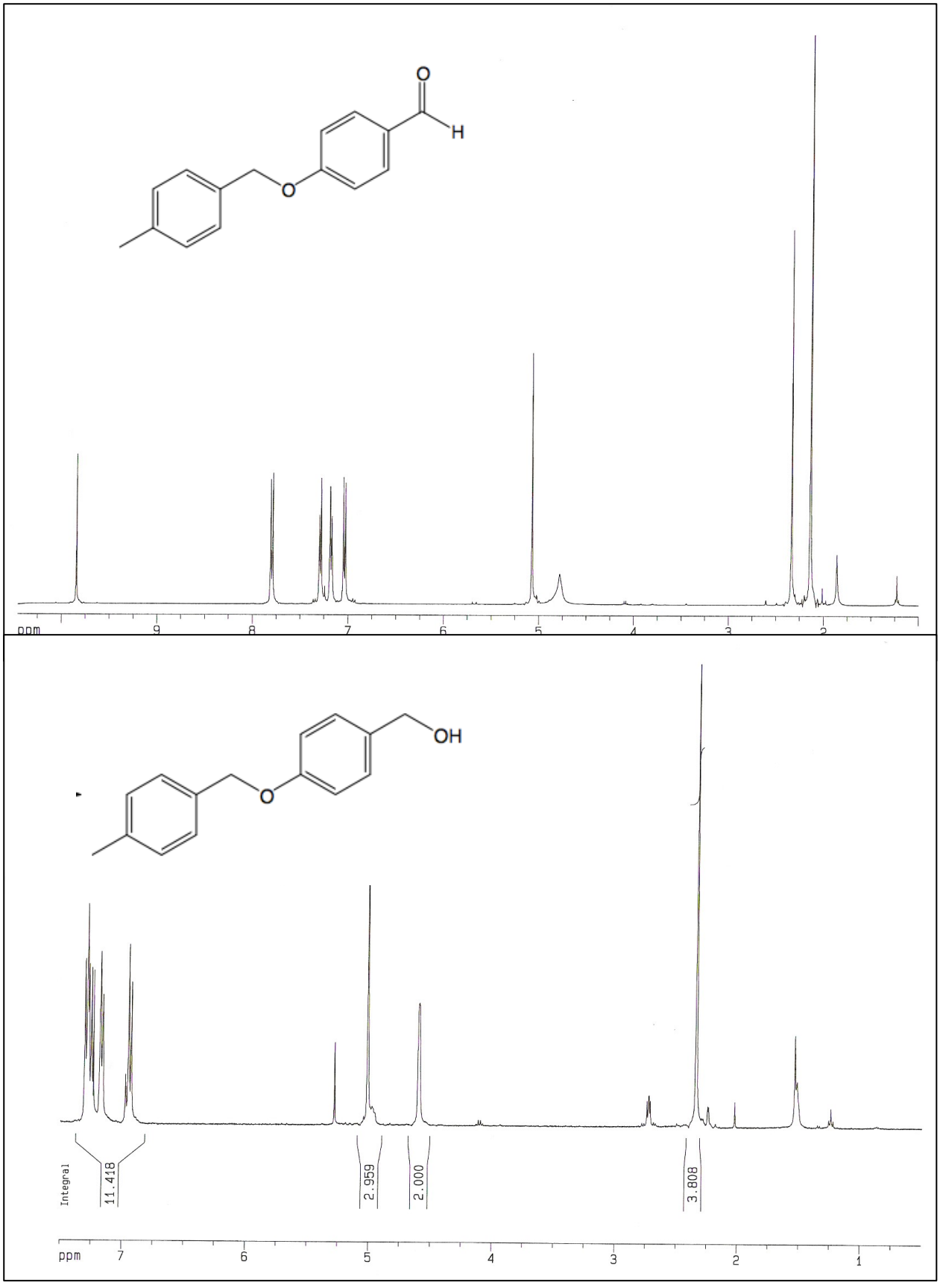
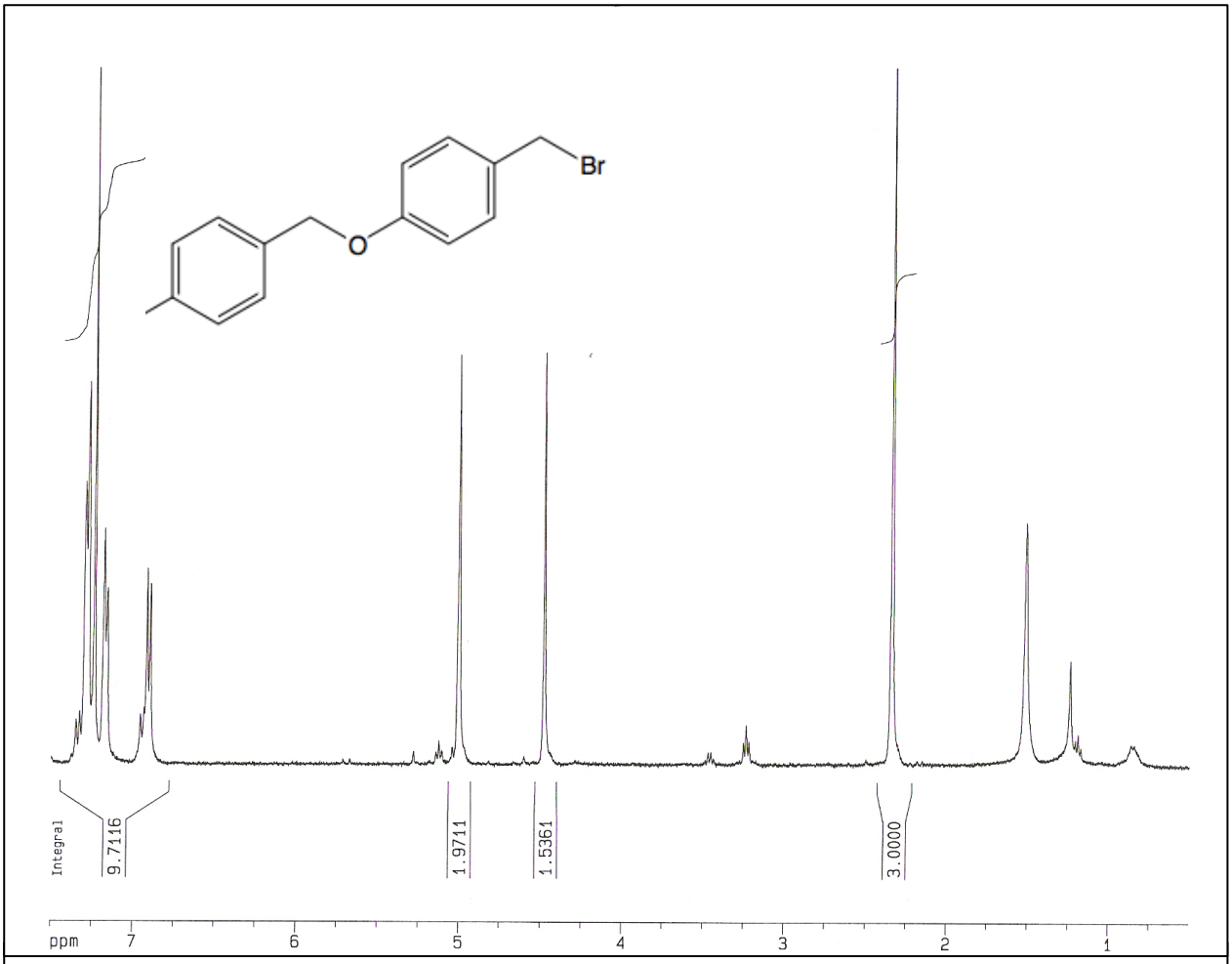
APPENDIX



Plot of phenol standard solutions used for TMSI cleavage reaction assay.

^{1}H NMR Spectra

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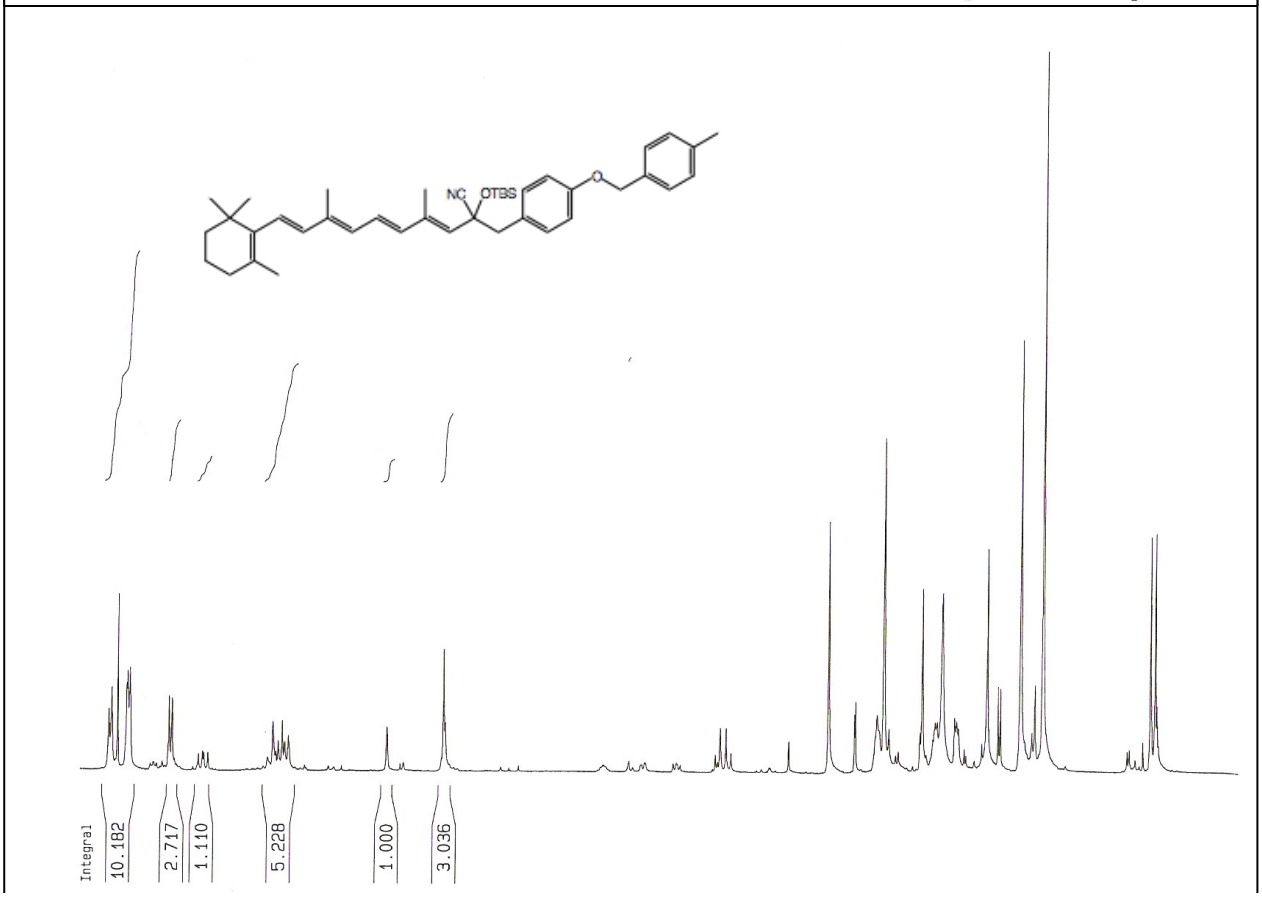
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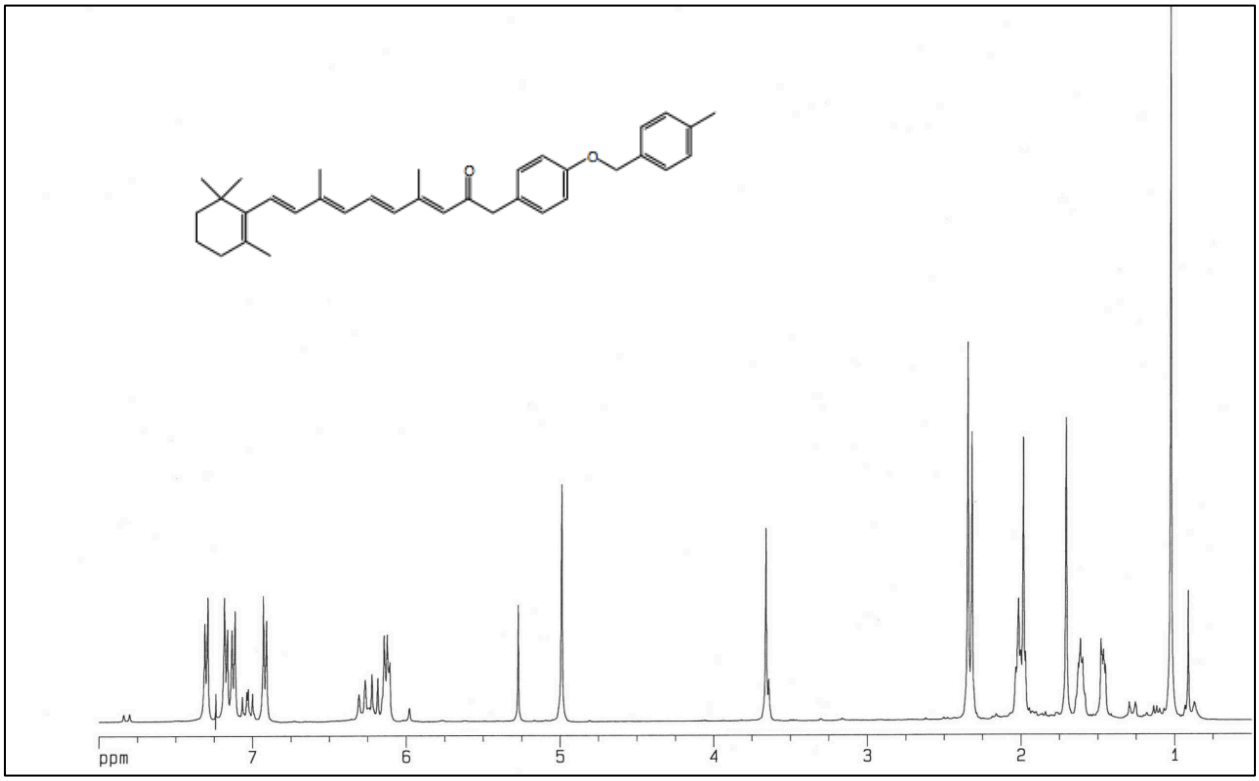
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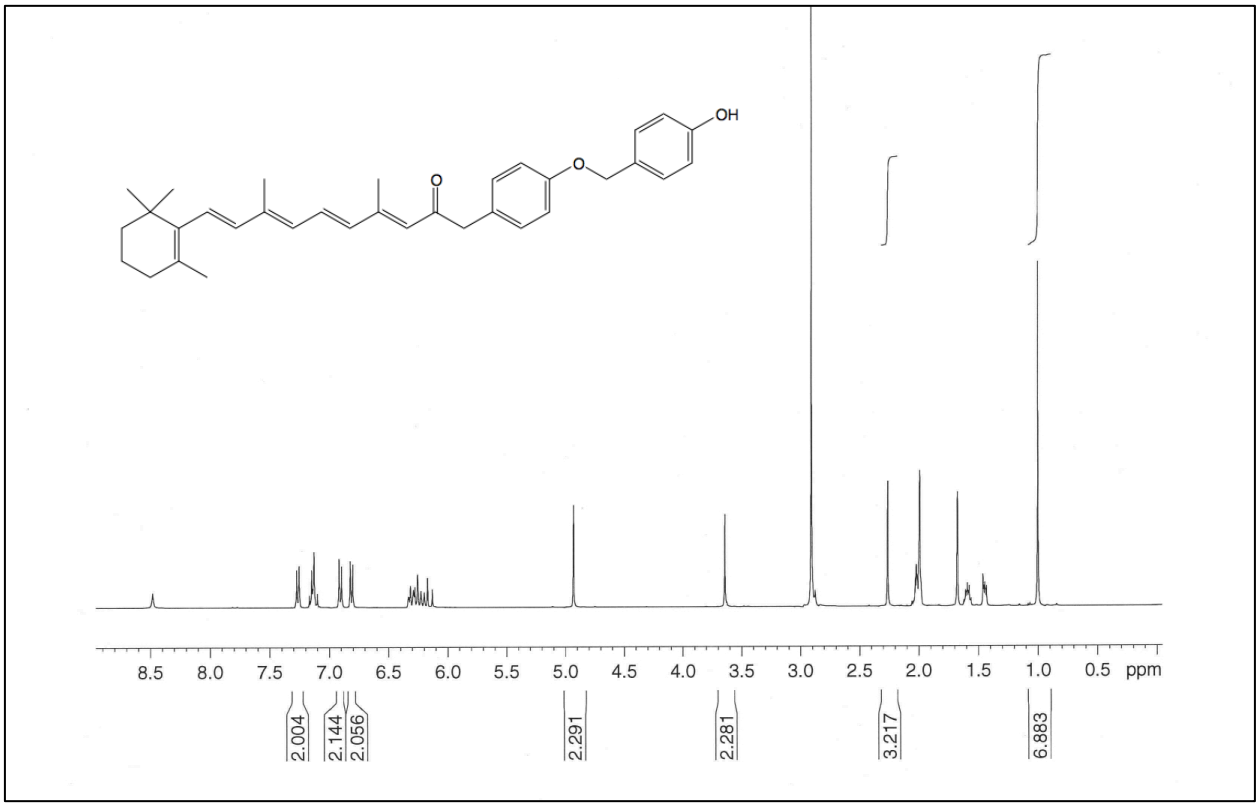
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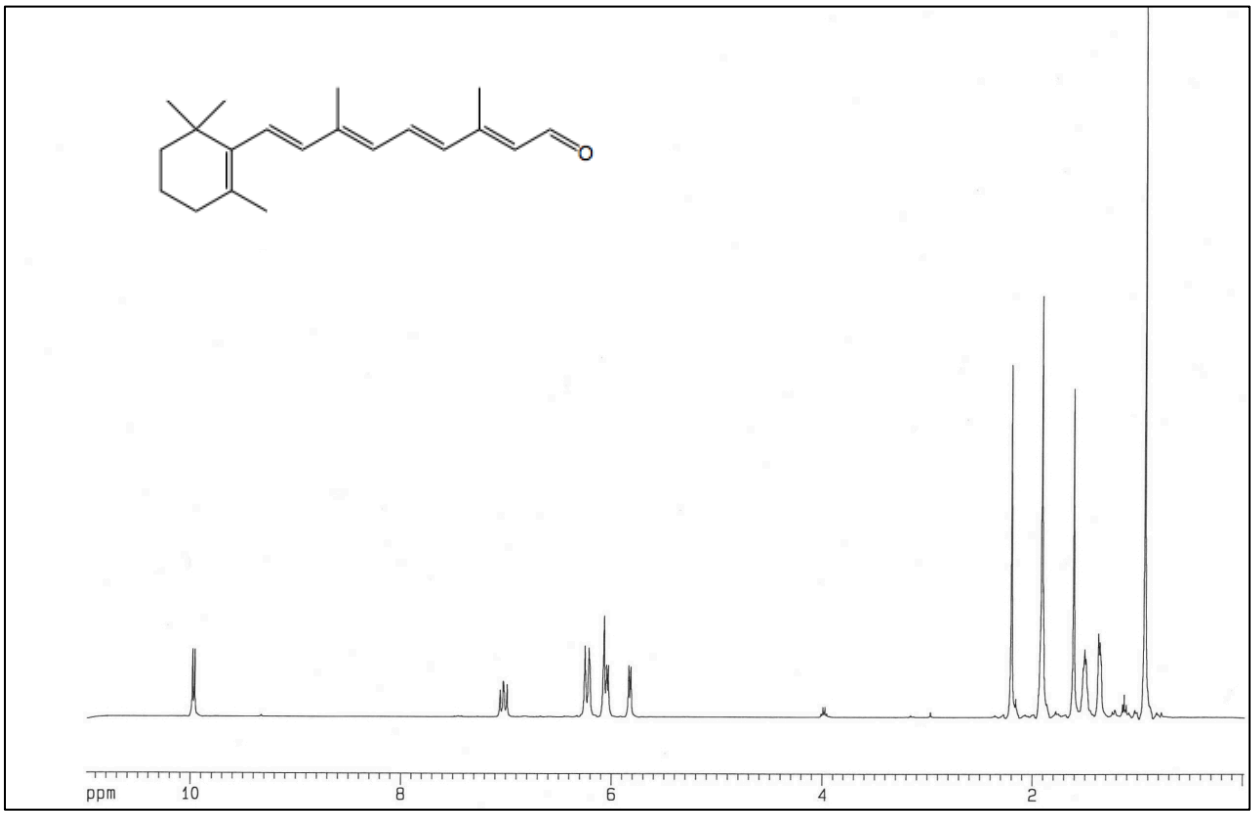
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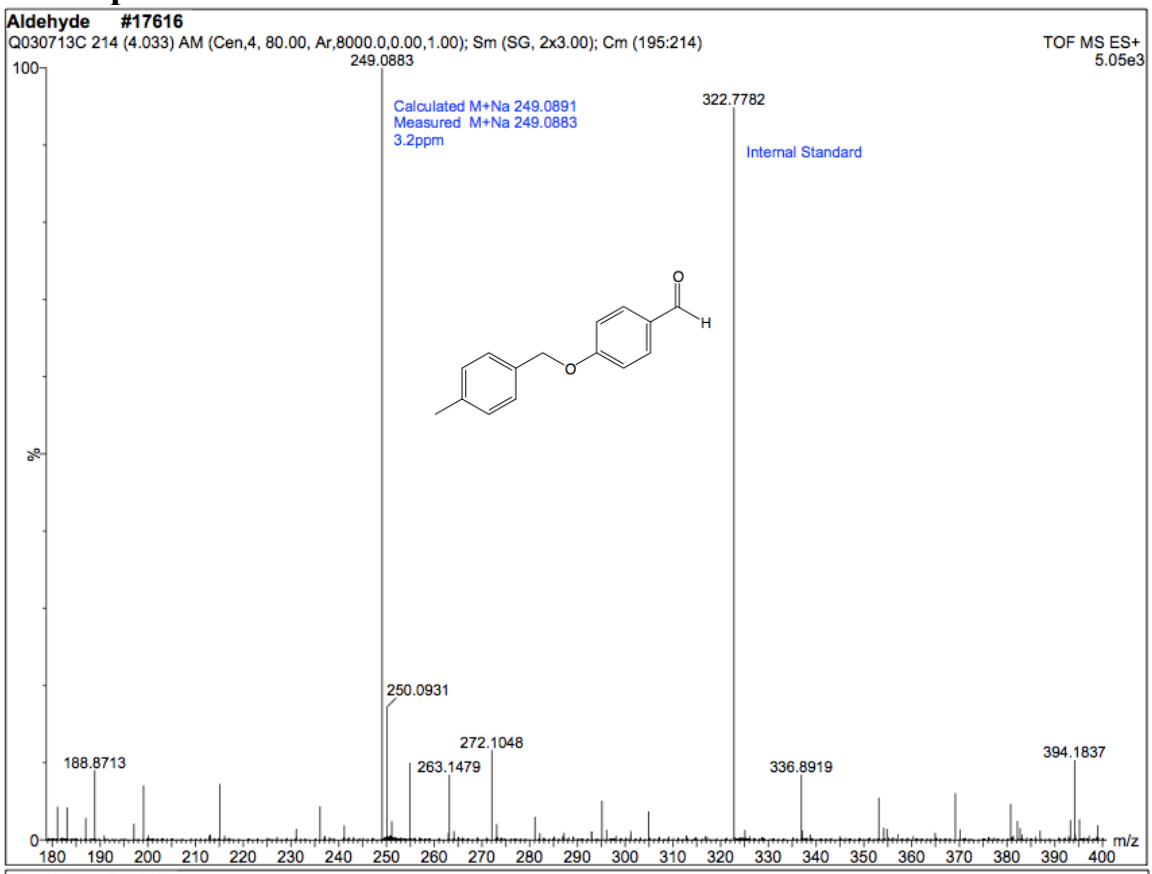

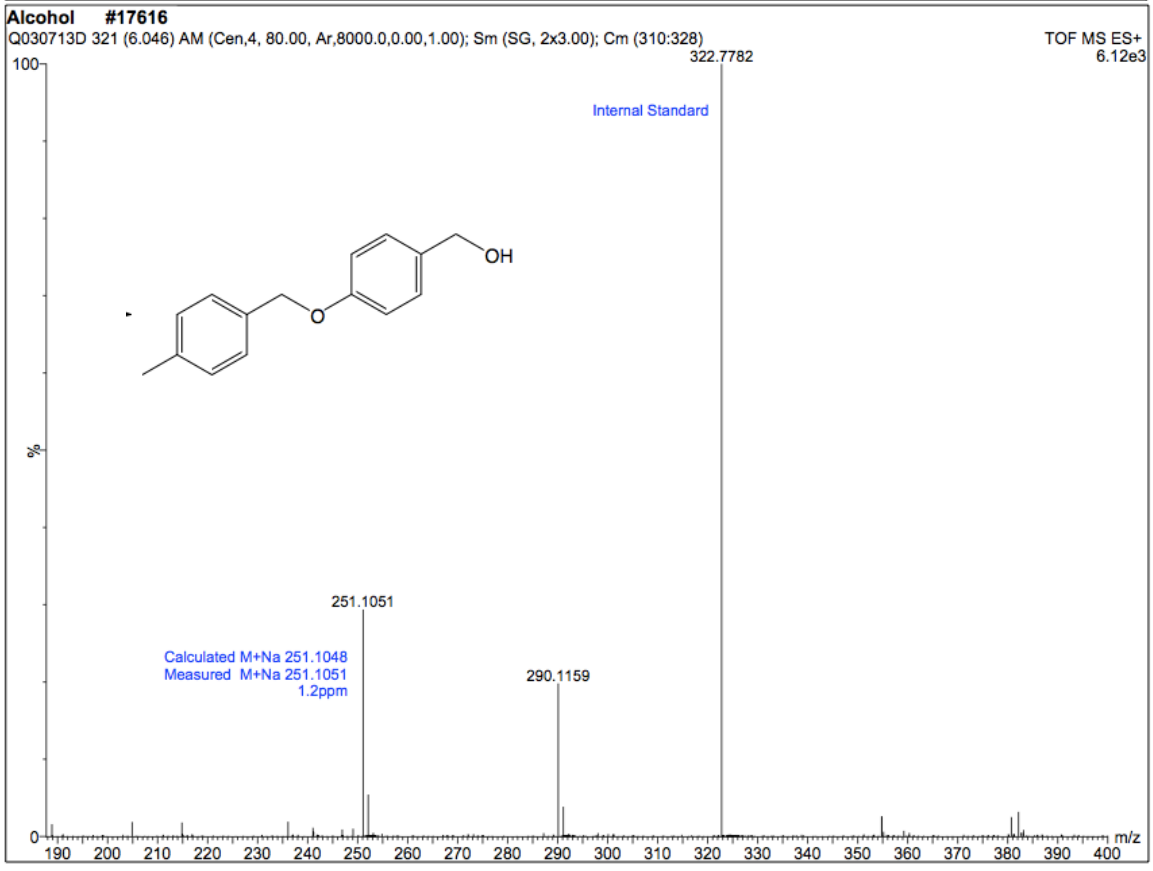

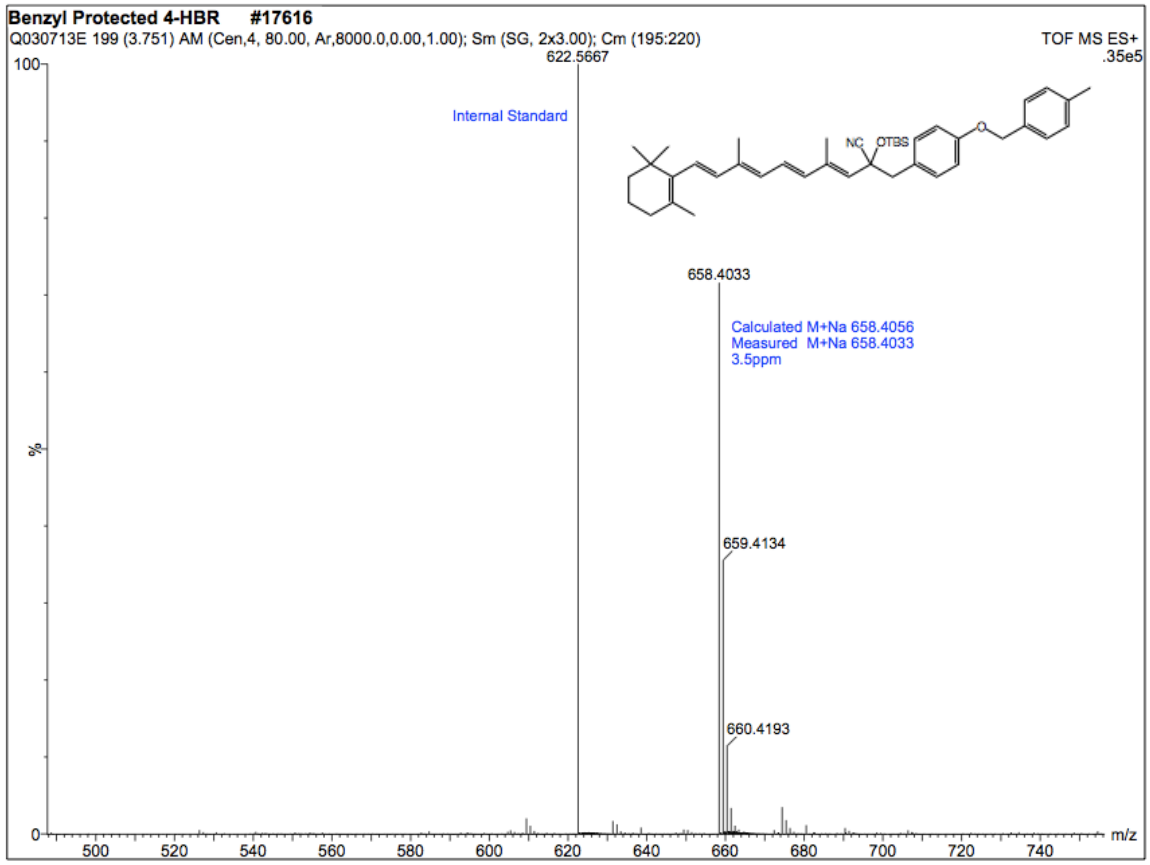
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Internal Standard
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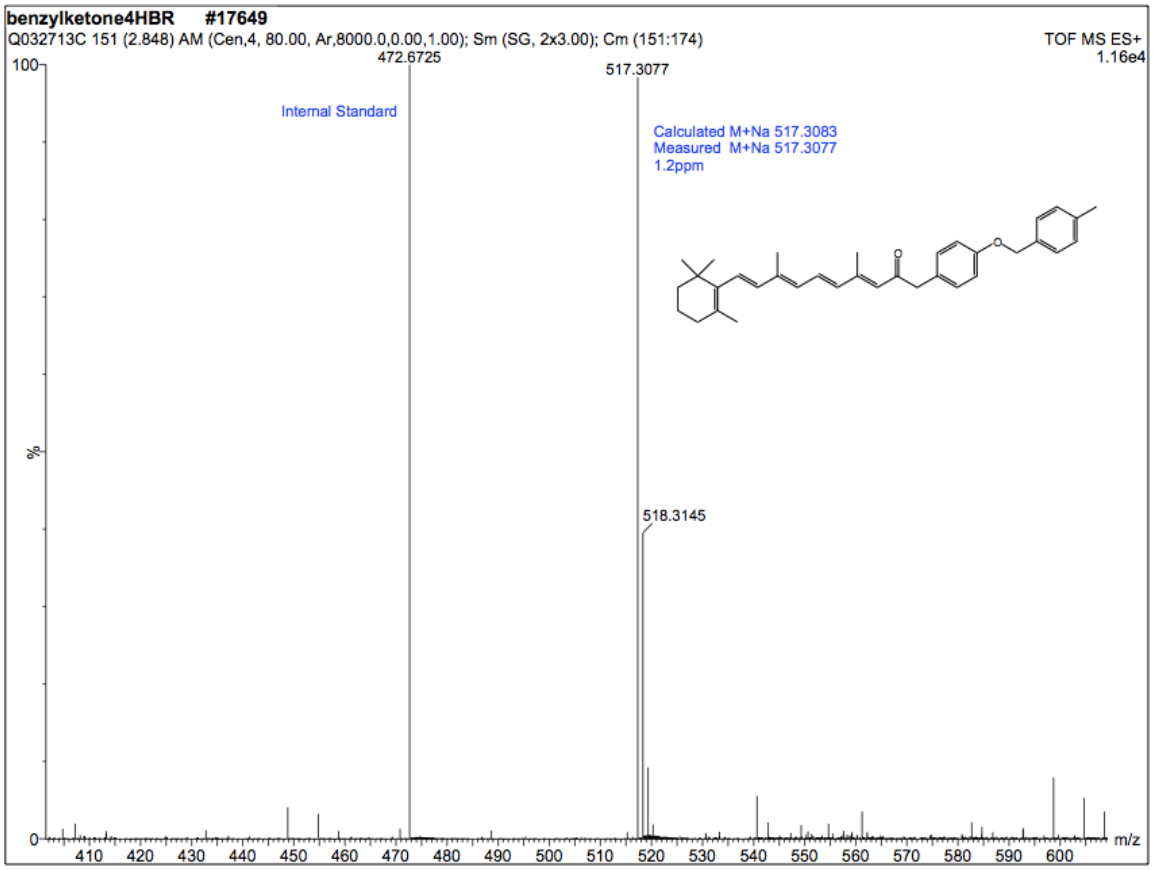
%
m/z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Internal Standard
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%
m/z